

2006

Some biochemical studies on the human lens nucleus

Xiaojia Wei
University of Wollongong

Follow this and additional works at: <https://ro.uow.edu.au/theses>

University of Wollongong

Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

Recommended Citation

Wei, Xiaojia, Some biochemical studies on the human lens nucleus, MSc thesis, Department of Chemistry, University of Wollongong, 2006. <http://ro.uow.edu.au/theses/574>

NOTE

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

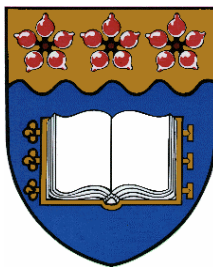
You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

SOME BIOCHEMICAL STUDIES ON THE HUMAN LENS NUCLEUS

Wei Xiaojia (Eric), B.Sc., M.Sc.

This thesis is presented as full requirements for the award of a
Master of Science by Research (Medicinal Chemistry)



Supervisor: Professor Roger Truscott

Department of Chemistry

University of Wollongong

Wollongong, Australia

March, 2006

CERTIFICATION

I, Xiaojia Wei, declare that this thesis, submitted in full fulfillment of the requirements for the award of Master of Science by Research, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Xiaojia Wei

29/03/2006

ACKNOWLEDGEMENTS

I would like to thank everyone who helped me throughout the duration of this research project.

To my supervisor, Professor Roger Truscott, for his advice, support and guidance, and the valuable time spent helping me.

To Dr John Korth, Dr Todd Mitchell, Assoc. Prof. Will Price and Prof. Tony Hulbert, for their patience, support and help.

To Anastasia, Ines, Jane, Karl, Madge, Michael, Nicole, Peter, Yoke and all past and present members of the Truscott research group, their support and assistance was much appreciated, and also their friendship.

To the Chemistry Department, the social nature of the department provides a very comfortable surrounding to work in.

To my friends Gareth and Carolyn for proof reading.

To my family, for their support, encouragement and endless love.

TABLE OF CONTENTS

TITLE PAGE.....	ERROR! BOOKMARK NOT DEFINED.
CERTIFICATION	II
ACKNOWLEDGEMENTS.....	III
TABLE OF CONTENTS	IV
LIST OF ABBREVIATIONS	VII
ABSTRACT.....	IX
CHAPTER 1: GENERAL INTRODUCTION	1
1.1. THE HUMAN LENS.....	1
<i>1.1.1. DEVELOPMENT OF THE HUMAN LENS.....</i>	<i>2</i>
<i>1.1.2. COMPOSITION OF THE HUMAN LENS</i>	<i>3</i>
1.1.2.1. Proteins in the Lens.....	3
1.1.2.2. UV-Filters.....	4
1.1.2.3. Antioxidants.....	5
1.2. HUMAN CATARACT	6
<i>1.2.1. CATEGORISING CATARACT TYPE</i>	<i>7</i>
<i>1.2.2. AGE-RELATED NUCLEAR CATARACT (ARNC).....</i>	<i>8</i>
<i>1.2.3. THE LENS BARRIER</i>	<i>9</i>
CHAPTER 2: DIFFUSION IN THE LENS.....	11
2.1. INTRODUCTION.....	11
<i>2.1.1. PHYSICAL PROPERTIES CHANGE WITH AGE</i>	<i>11</i>
<i>2.1.2. DIFFUSION</i>	<i>11</i>
<i>2.1.3. PURPOSE AND AIMS</i>	<i>13</i>
2.2. EXPERIMENTAL	14
<i>2.2.1. MATERIALS</i>	<i>14</i>
2.2.1.1. Biological Samples.....	14
2.2.1.2. Chemicals and Solutions.....	14
<i>2.2.2. APPARATUS</i>	<i>15</i>
2.2.2.1. Modified Franz Cell.....	15
2.2.2.2. Liquid Scintillation Counter	16

2.2.3. PROCEDURES.....	17
2.2.3.1. Lens Tissue Specimens Preparation	17
2.2.3.2. Permeability Experiment.....	18
2.3. RESULTS AND DISCUSSION	19
 CHAPTER 3: OXYGEN CONSUMPTION IN THE LENS.....	22
3.1. INTRODUCTION.....	22
3.1.1. OXIDATIVE STRESS.....	22
3.1.2. OXYGEN CONSUMPTION IN THE LENS	23
3.1.2.1. Mitochondria	24
3.1.2.2. Mitochondrial Oxygen Consumption	25
3.1.2.3. Non-mitochondrial Oxygen Consumption in the Lens	27
3.1.3. OXYGEN ANALYZER – CLARK (PO ₂) ELECTRODE	29
3.1.4. RESEARCH AIMS.....	30
3.2. EXPERIMENTAL	31
3.2.1. CHEMICALS AND SOLUTIONS.....	31
3.2.2. BIOLOGICAL MATERIALS	32
3.2.3. GENERAL EQUIPMENT.....	33
3.2.4. PSH AND PROTEIN ASSAY.....	33
3.2.5. MEASUREMENTS OF PSH OXYGEN CONSUMPTION	35
3.2.5.1. O ₂ Consumption	35
3.2.5.2. Non-mitochondrial Oxygen Consumption	37
3.3. RESULTS AND DISCUSSION	38
3.3.1. THE EFFECT OF FREEZING.....	38
3.3.2. PROTEIN SULPHYDRYL IN THE LENS CORE AS POTENTIAL OXYGEN CONSUMER	39
3.3.3. FUTURE WORK	41
3.4. CONCLUSIONS	41
 CHAPTER 4: CHOLESTEROL QUANTIFICATION.....	43
4.1. INTRODUCTION.....	43
4.1.1. MEMBRANE LIPIDS.....	43
4.1.2. CHOLESTEROL	44
4.1.3. CHOLESTEROL IN THE LENS.....	45
4.1.4. CHOLESTEROL AND AGING OF THE LENS	46
4.1.5. MASS SPECTROMETRY	48
4.1.6. PROJECT AIMS	52
4.2. EXPERIMENTAL	52
4.2.1. CHEMICALS AND MATERIALS	52
4.2.2. LIPID EXTRACTION.....	53
4.2.3. DI/EI MASS SPECTROMETRY	54
4.2.4. INTERNAL STANDARD.....	55
4.2.5. CHOLESTEROL QUANTIFICATION.....	57

4.2.5.1. Standard Curve	57
4.2.5.2. Cholesterol Quantification	58
4.3. RESULTS AND DISCUSSION	58
<i>4.3.1. STANDARD CURVE USED FOR DETERMINATION OF CHOLESTEROL QUANTIFICATION</i>	<i>58</i>
<i>4.3.2. CHOLESTEROL IN MAMMALIAN LENSES</i>	<i>59</i>
<i>4.3.3. DETERMINATION OF CHOLESTEROL LOSSES DURING EXTRACTION</i>	<i>63</i>
<i>4.3.4. RECOVERY OF INTERNAL STANDARD (DEUTERATED CHOLESTEROL).....</i>	<i>66</i>
<i>4.3.5. CHOLESTEROL IN THE HUMAN LENS.....</i>	<i>67</i>
4.4. CONCLUSIONS	69
 APPENDIX A	 71
 APPENDIX B.....	 72
 APPENDIX C.....	 73
 APPENDIX D.....	 74
 APPENDIX E	 75
 APPENDIX F	 76
 LIST OF REFERENCES	 77

LIST OF ABBREVIATIONS

The following abbreviations were used in this thesis

ACRF	Australian Cataract Research Foundation
ADP	Adenosine Diphosphate
Ag/AgCl	Silver/Silver Chloride
AHBG	4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid glucoside
AQP0	Aquaporin0
ARNC	Age-related Nuclear cataract
ATP	Adenosine Triphosphate
BCA	Bicinchoninic Acid
BSA	Bovine Serum Albumin
CHCl ₃	Chloroform
CoQ	CoenzymeQ
CytC	CytochromeC
DC Voltage	Direct Current Voltage
DI	Direct insertion
DNP	2,4-dinitrophenol
DTNB	5,5'-dithiobis-(nitrobenzoic acid)
EDTA	Ethylenediaminetetraacetic Acid
EI Ionization	Electron Impact Ionization
ESI	Electrospray Ionization
ETC	Electron Transport Chain
FCCP	Carbonylcyanide-p-trifluoromethoxyphenyl hydrozone
GSH	Glutathione (reduced form)
GSSG	Glutathione (oxidized form)
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMM	High Molecular Mass
HPLC	High Performance Liquid Chromatography
IAA	Iodoacetic Acid
IOL	Intraocular Lens
KCl	Potassium Chloride
Kyn	Kynurenine
MALDI	Matrix Assisted Laser Desorption Ionization
MeOH	Methanol
m/z	Mass to Charge
NADH	Nicotinamide Adenine Dinucleotide (reduce form)

NMR	Nuclear Magnetic Resonance
O ₂	Molecular Oxygen/Oxygen
PBS	Phosphate-buffered Saline
pO ₂	Partial Pressure of Oxygen
PSH	Peotein Sulphydryl
R ²	Regression
Rf Voltage	Radio Frequency Oscillation Voltage
ROS	Reactive Oxygen Species
SDS	Sodium Dodecyl Sulphate
SIM	Selected Ion Monitoring
TCA	Trichloracetic Acid
Tris-HCl	Tris(hydroxymethyl)aminomethane
UV	Ultra Violet
WR	Working Reagent
WS	Water Soluble
y.o.	Years Old

ABSTRACT

Barrier¹⁶ formation has been shown to occur in the lens with age. It is important to understand the physiological changes in the lens that occur upon the formation of the barrier and their implication on the onset of cataract and presbyopia (old man's eyes). In this study, three factors related to the formation of the barrier were investigated: diffusion rate changes in the lens nucleus, oxygen consumption and cholesterol compositional changes in the lens with age.

A Franz Cell was used to measure the diffusion rate changes in the nucleus of the human lens. No significant differences in the rate of diffusion between young and old lenses could be detected with this technique.

The role of protein sulphydryls as secondary oxygen consumers was also studied. It was shown that protein sulphydryls reacted readily with oxygen, suggesting that protein sulphydryls are a secondary O₂-consumption system in the center of the lens. Mitochondria are the primary oxygen consumers in the lens.

A technique for the quantification of cholesterol in lipid extracts was developed. Results obtained were comparable to published results using traditional methods. The concentration of cholesterol in the young human lens was found to be approximately 3-fold greater than that of the bovine, ovine and porcine lenses, and ~5 times greater

than in the gallinaceous lens. These differences were even more pronounced when an elderly human lens was examined. The nucleus of the human lens was found to have a higher level of cholesterol content than that in the cortex and the concentration of cholesterol also exhibited a significant increase with age in both nuclear and barrier regions.